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Sequential morphological and functional changes in kaolin-induced hydrocephalus

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An experimental model of kaolin-induced hydrocephalus in the dog was studied in order to evaluate the progress of ventricular dilatation and the communications between the ventricular system and the subarachnoid space. Skull and spine radiological studies were obtained after metrizamide intraventricular injection, and the baseline ventricular pressure and cerebral pulse pressure amplitude were measured in anesthetized animals. Intracranial compliance and resistance to drainage of cerebrospinal fluid were calculated by means of bolus injection test. Light and scanning electron microscope studies were done at different developmental stages of hydrocephalus. With these experimental parameters, two successive phases were seen: an initial acute hypertensive hydrocephalus (H1) with high resistance, low compliance, severe ependymal damage, and subependymal edema; and a late chronic normotensive hydrocephalus (H2) with little resistance increase, normal compliance, epithelial regeneration, and subependymal gliosis. Both the H1 and H2 stages showed an increase in the cerebral pulse pressure amplitude.

KEY WORDS • **experimental hydrocephalus** • **kaolin** • **cerebrospinal fluid** • **ventriculography** • **ependyma**

INTRACISTERNAL administration of kaolin in the experimental animal produces an obstructive hydrocephalus and dilatation of the spinal cord central canal. These changes are due to adhesive arachnoiditis of the basal cisterns and obstruction of the fourth ventricle outlets.[8,10,17,25,40] In kaolin-induced hydrocephalus models, two successive phases have been described; namely, an initial hypertensive hydrocephalus stage (H1) followed by a condition of chronic normotensive hydrocephalus due to development of compensatory channels (H2).[11,18,20,32,33]

The aim of this work has been to study sequentially the development of hydrocephalus induced in an animal model by intracisternal kaolin injection. We have used radiological studies, observations of cerebrospinal fluid (CSF) dynamics, and pathological examination with the intention of determining the nature of the compensatory systems.

Materials and Methods

A total of 38 adult mongrel dogs, averaging 16 kg each in weight, were used for this study. The animals were anesthetized with sodium thiopental (10 mg/kg) and atropine (0.02 mg/kg), and were maintained with spontaneous ventilation. Experimental hydrocephalus was achieved by intracisternal injection of sterile kaolin solution (50 mg/kg). In control animals, 3 cc saline was injected after cisternal puncture.

In order to evaluate the progression of hydrocephalus, vertex and lateral skull and spine x-ray films were taken after intraventricular injection of 2 cc metrizamide (Amipaque). The ratio of the maximum distance between both temporal horns (T) to the maximum distance between the inner skull tables (S), the T/S ratio, was chosen as the ventriculographic index. Other radiological findings, such as the spread of the radiolabeled contrast material to the subarachnoid space in the posterior fossa or spine, and the filling and size of the lateral recesses of the fourth ventricle were also considered. Ventriculography was performed in five normal dogs, and 7 to 15 days after kaolin injection in five animals, 16 to 20 days after injection in three, 21 to 30 days after injection in three, and more than 30 days post-injection in three experimental animals.

Studies of intracranial pressure, using CSF dynamics and recording the baseline ventricular pressure (VP_0),

were performed in anesthetized animals.* The CSF pulse pressure amplitude (Amp) was measured at baseline, and an Amp/VP_0 index was calculated. Intracranial compliance and resistance to CSF outflow were also measured by means of a bolus injection test,[28] using manual intraventricular injections of 0.05 to 0.5 cc saline. The manometric study was done between 7 and 15 days after kaolin injection in three animals, between 16 and 20 days after injection in four, between 21 and 30 days after injection in three, and more than 30 days post-injection in four animals. A manometric study was also performed in five normal dogs.

In all animals, the behavioral changes and neurological deficits that appeared after experimental procedures were recorded daily. The animals were sacrificed between 2 and 17 weeks after cisternal injection, and the brain, brain stem, and cervical spinal cord were removed. The specimens were fixed in 10% formalin and stored for histological processing.

Six experimental and four control animals were used for scanning electron microscopy (SEM) study. Three experimental and two control animals were sacrificed 14 and 60 days after cisternal injection. In experimental animals, presacrifice CSF dynamic studies and postmortem ventricular measurements were made to verify the hydrocephalus development stage. Fixation was performed *in vivo* in anesthetized animals by means of an intraventricular perfusion of 100 cc 1.5% glutaraldehyde solution at 100 cm H_2O hydrostatic pressure. The brain was removed, and 0.5 × 0.5-cm blocks of the external wall were taken from the left lateral ventricle at the atrial level. The specimens were prepared for SEM study and examined. Routine light microscopic examinations of the ventricular system and cervical central canal of the spinal cord were also done.

Results

Clinical Observations

Eight to 10 days following kaolin injection, the experimental animals showed obvious deterioration in their general condition, with timidness, lethargy, anorexia, and ataxic gait. However, after 1 month the animals returned to an apparently normal stage, although weight loss persisted.

Manometric Studies

Table 1 summarizes the mean values of manometric parameters in control and experimental hydrocephalic animals. The results varied depending on the interval after kaolin injection. In the control group, the mean VP_0 was 6.4 ± 1.6 mm Hg. In H1 hydrocephalic animals the mean value of VP_0 was 22.8 ± 9.2 mm Hg ($p < 0.01$) and in H2 animals the mean value of VP_0 was 4.9 ± 2.7 mm Hg ($p < 0.30$). The VP_0 mean value for the H1 group was significantly higher than that for the H2 measurements ($p < 0.02$).

TABLE 1

*Manometric parameters in control and experimental hydrocephalic groups**

Parameters	Control Group	Experimental Groups	
		H1	H2
no. of animals	5	7	7
mean days after kaolin injection	—	17	49
VP_0 (mm Hg)	6.4 ± 1.6	22.8 ± 9.2	4.9 ± 2.7
C (ml/mm Hg) ($\cdot 10^{-3}$)	17.5 ± 5.6	6.7 ± 2.0	23.2 ± 6.7
R (mm Hg/ml/min)	22.8 ± 9.1	78.8 ± 37.7	40.5 ± 11.0
Amp (mm Hg)	1.0 ± 0.5	4.9 ± 3.4	2.8 ± 0.4
Amp/VP_0 index	0.17 ± 0.11	0.21 ± 0.13	0.84 ± 0.41

* VP_0 = basal ventricular pressure; C = compliance; R = resistance; Amp = cerebrospinal fluid pulse pressure amplitude; H1 = acute stage of hydrocephalus; H2 = chronic stage (see text). Values are means ± standard error of the mean.

Mean control group compliance was $(17.5 \pm 5.6) \cdot 10^{-3}$ ml/mm Hg. In H1 hypertensive animals, the compliance decreased, with a mean value of $(6.7 \pm 2.0) \cdot 10^{-3}$ ml/mm Hg ($p < 0.001$), whereas in the H2 normotensive animals mean compliance normalized, with a value of $(23.2 \pm 6.7) \cdot 10^{-3}$ ml/mm Hg ($p < 0.30$). The resistance in the control group was 22.8 ± 9.1 mm Hg/ml/min, and was increased in the experimental H1 hypertensive stage, with a mean value of 78.8 ± 37.7 mm Hg/ml/min ($p < 0.05$). In the late hydrocephalus series (H2) the mean resistance value continued to be slightly increased (40.5 ± 11.0 mm Hg/ml/min) in comparison with control animals ($p < 0.05$), but not with the H1 group ($p < 0.20$).

The mean CSF pulse pressure amplitude was 1.0 ± 0.5 mm Hg in control animals. It was much higher in the H1 (4.9 ± 3.4 mm Hg, $p < 0.05$) and H2 experimental stages (2.8 ± 0.4 mm Hg, $p < 0.01$). No differences appeared between both experimental groups (Fig. 1). Finally, the mean Amp/VP_0 index was 0.17 ± 0.11 in control animals and 0.21 ± 0.13 at the H1 hypertensive stage ($p < 0.80$). At the H2 normotensive stage, the mean Amp/VP_0 index was 0.84 ± 0.41, with differences in respect to the control group ($p < 0.02$) and the experimental H1 hypertensive animals ($p < 0.05$).

Ventriculographic Findings

The results were analyzed comparing the two experimental series. The acute hydrocephalic (H1) animals had ventriculography performed on average 13 days after kaolin injection, and the chronic hydrocephalic (H2) series underwent radiological study after a mean interval of 42 days. In both series, the mean T/S value was significantly higher than in normal animals (normal 0.66 ± 0.02; H1 0.71 ± 0.03, $p < 0.02$; H2 0.71 ± 0.02, $p < 0.05$), whereas there were no differences in the T/S ratio between the H1 and H2 series ($p < 0.60$).

Generally, in experimental hydrocephalic animals,

* Model 1280 pressure transducer manufactured by Hewlett-Packard Co., Waltham, Massachusetts.

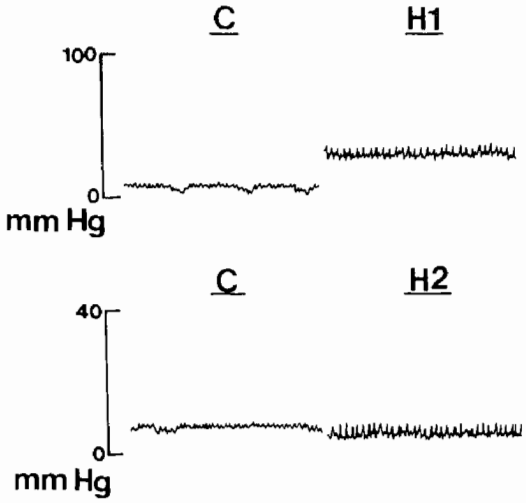

FIG. 1. Baseline ventricular pressure (VP_0) in control (C) and experimental animals (H1, H2). In both the H1 and H2 hydrocephalus models the cerebral pulse pressure amplitude is higher than in the control animals, although the VP_0 is normal in H2 animals.

no radiolabeled contrast material spread from the ventricular system to the posterior fossa cisterns, and the lateral recesses of the fourth ventricle were enlarged in comparison with those of normal animals (Fig. 2). However, in five (33%) hydrocephalic animals a small amount of metrizamide was detected in the posterior fossa subarachnoid space, and in two cases (13%) the lateral recesses were not enlarged. All experimental animals examined showed metrizamide filling of the spinal cord central canal, although in no case was the spread of contrast material to the spinal subarachnoid space recorded. Three morphological patterns were observed: in three cases (21%) there was filamentous and regular filling of spinal cord central canal, in two animals (13%) the central canal was uniformly dilated, and in 10 cases (66%) communicating intramedullary cavities appeared along the spinal cord, in particular at the cervical level. No differences in appearance existed between the H1 and H2 groups.

Pathological Findings

In the control group, light microscopic study of the ventricular wall and spinal cord showed a normal ependymal epithelium and an open spinal cord central canal. The ventricular wall studied on SEM showed small irregular microvilli and large cilia. The cilia were grouped in clusters over each apical pole of the ependymal cells. The microvilli covered this ventricular surface, but were concentrated near the cell border (Fig. 3).

Microscopic study of the ependymal wall in animals 14 days after kaolin injection showed severe ependymal damage and subependymal edema. The SEM study of the ependymal surface showed massive loss of cilia and some rounded and unciliated cells (Fig. 4). In the hydrocephalic animals studied 60 days after kaolin injection, an attempt at ependymal regeneration was observed. There was chronic edema and reactive gliosis at the subependymal level. Scanning electron micrographic examination demonstrated nearly normal cilia grouped in clusters, although these were more separated than in control animals (Fig. 5). In experimental animals there was a dilatation of the spinal cord central canal, with ependymal damage and slight subependymal edema. More severe lesions could appear, with complete disappearance of epithelium, massive subependymal edema, and necrotic cavities. In the latter

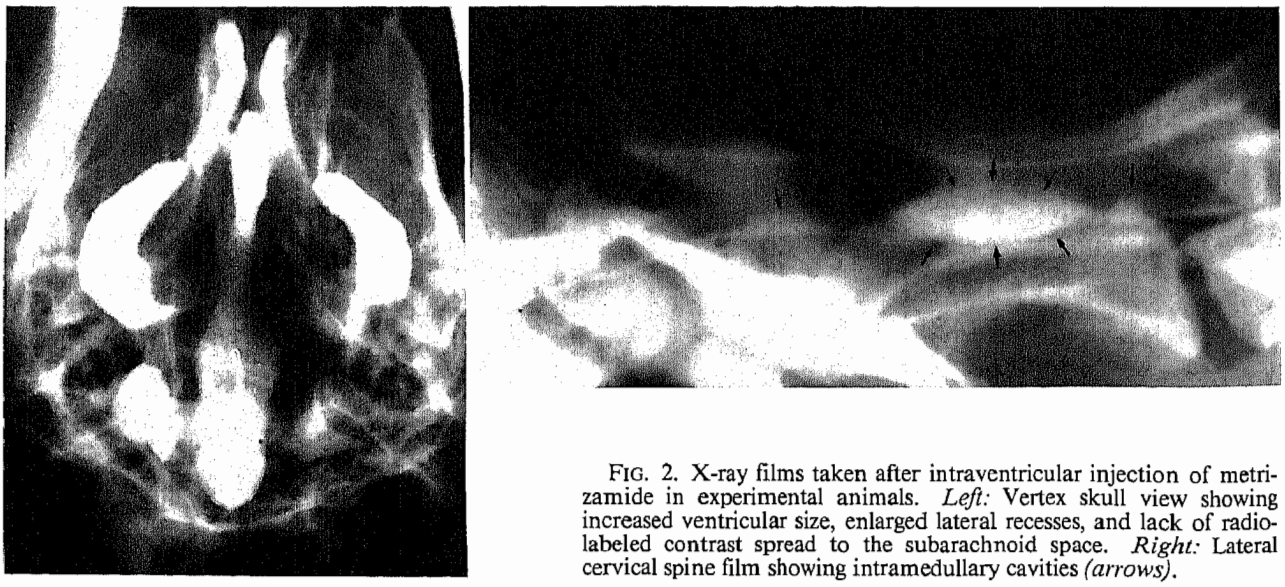

FIG. 2. X-ray films taken after intraventricular injection of metrizamide in experimental animals. *Left:* Vertex skull view showing increased ventricular size, enlarged lateral recesses, and lack of radiolabeled contrast spread to the subarachnoid space. *Right:* Lateral cervical spine film showing intramedullary cavities *(arrows)*.

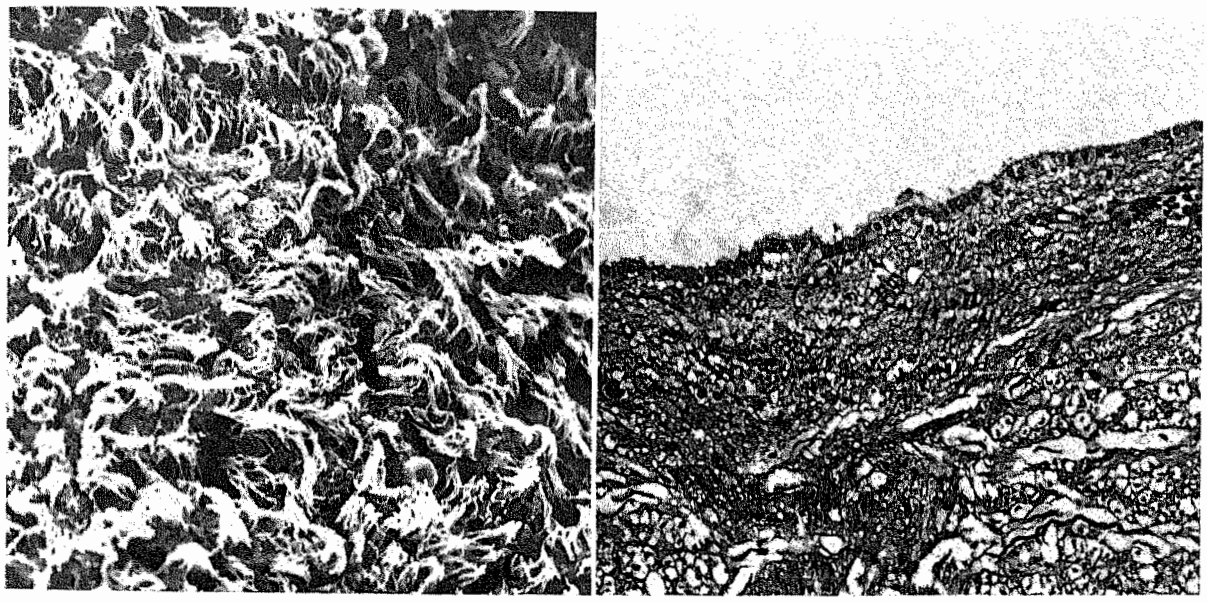

FIG. 3. Scanning electron micrograph (× 500, *left*) and photomicrograph (H & E, × 100, *right*) showing normal appearance of the ependyma of the lateral ventricle of a control animal.

phases, when cavities formed, they were surrounded by necrotic tissue with glial activity and absence of wall.

Discussion

In this experimental study, both acute (H1) and chronic (H2) stages of kaolin-induced hydrocephalus development have been described. The initial phase follows a course similar to hypertensive hydrocephalus, with an increase in resistance to CSF drainage and a decrease of compliance, whereas later the ventricular pressure and compliance are normalized with maintenance of ventriculomegaly. The CSF resistance in this chronic stage is slightly higher than in the control group.

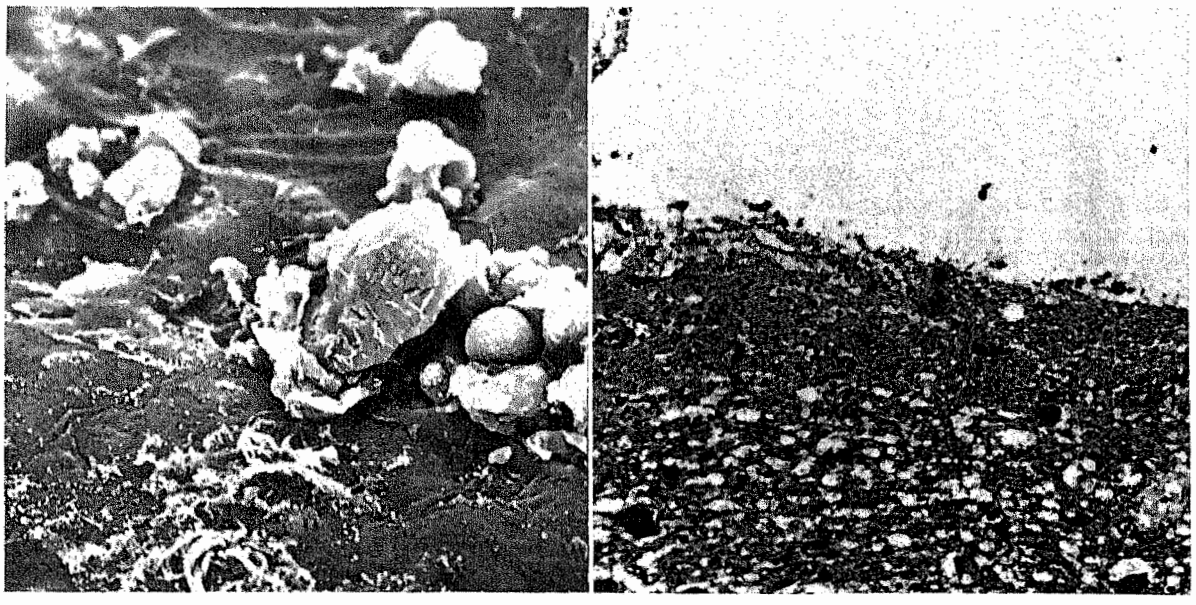

FIG. 4. Ependyma of the lateral ventricle of a hydrocephalic animal 14 days after intracisternal kaolin injection. The animal was in the early hypertensive hydrocephalus (H1) stage. *Left:* Scanning electron micrograph showing some damaged ciliary clusters and small rounded cells. × 1500. *Right:* Photomicrograph showing severe ependymal damage and subependymal extracellular edema. H & E, × 100.

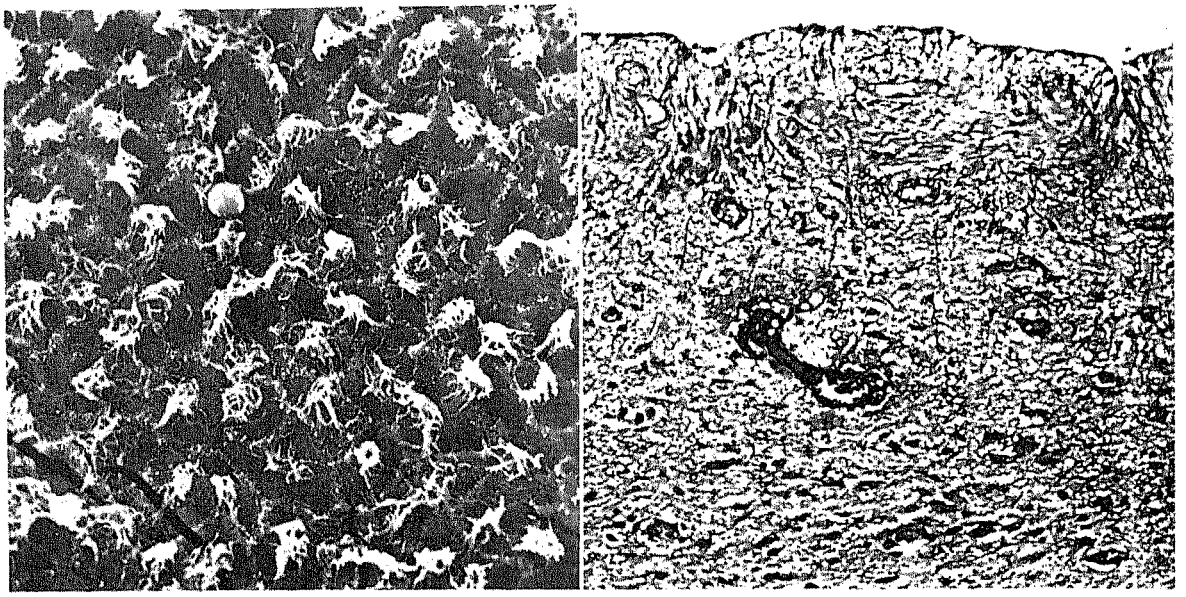

FIG. 5. Ependyma of the lateral ventricle of a hydrocephalic animal 60 days after intracisternal kaolin injection. The animal was in the normotensive hydrocephalus (H2) stage. *Left:* Scanning electron micrograph showing the ependymal surface with ciliary clusters more separated than in control animals. × 500. *Right:* Photomicrograph showing ependymal regeneration and subependymal gliosis and chronic edema. H & E, × 100.

The transition to a normal-pressure situation is possible through the development of compensatory systems which reduce the resistance to CSF drainage.

Intraventricular CSF absorption has been considered as an alternative pathway in experimental hydrocephalus, either by the transependymal route or through the choroid plexus.[22,24,26,31,32,38,39,42] This has been suggested by studies with dyes,[20,27,31] molecular or isotope tracers,[20,27,31,37] water content measurement in periventricular areas,[14,19,20] and recently by computerized tomography studies of periventricular density and its changes after metrizamide ventriculography or shunting.[10,34] However, many of the findings obtained with these techniques can be accounted for by diffusion mechanisms and they do not necessarily imply real bulk transfer of CSF.[12,13] Transventricular CSF absorption was also proposed in studies using ventricular perfusion techniques,[3,20] until the demonstration by Eisenberg, *et al.*,[12] that absorption in the isolated ventricular system of cats with kaolin-induced hydrocephalus is virtually nil.

On the other hand, ependymal and subependymal pathological findings in experimental hydrocephalus have been related to transependymal CSF absorption.[22,23,26,31,34,36,41–43] Ventricular wall changes observed in our experimental study are similar to those described in other experimental hydrocephalus models,[8,10,16,23,36] and they are probably nonspecific.

Some authors pointed out that an alternative compensatory mechanism is CSF absorption in the spinal subarachnoid space, where CSF spreads from the dilated central canal of the spinal cord.[12,13,25,35] In the rabbit, in which a communication between the spinal cord canal and subarachnoid space is observed even in normal conditions,[6] it has been shown that the ventricular size after intracisternal kaolin injection is related to the degree of obex obstruction by the secondary inflammatory reaction.[40] In cats with kaolin-induced hydrocephalus, communications between the spinal cord canal and subarachnoid space have been suggested in studies using dyes and radiological, radioisotope and perfusion techniques.[12,13,25,35]

Apart from exceptional cases,[1,16,35] it has not been possible to locate the exact points of communication. Our study suggests that, in the compensated hydrocephalic dog at least, the actual size of these channels must be small, although they permit the decrease in resistance to CSF outflow. The cerebral pulse pressure amplitude (Amp) remains high as in the acute hypertensive stage, probably because it is not damped in the subarachnoid space.[1,2,15,17]

Once CSF reaches the subarachnoid space, its absorption and entry into the vascular space are possible through the spinal arachnoid villi.[29,41] However, CSF is also drained via the lymphatic system.[4,5,7,29] The passage of CSF from the subarachnoid space into the lymphatic channels and nodes would be made through a nonarachnoid route,[30] anatomically located at the cranial and spinal nerve exit zones.[7,9,21]

Obviously, in animal species where the spinal cord central canal communicates with the spinal subarachnoid space, in normal or hydrocephalic conditions, the

development of this path would be the first compensatory system. Thus, this proposed mechanism is the main route of CSF drainage in obstructive hydrocephalus in the cat[1,12,13,25,35] and rabbit,[6,40] but it seems that in communicating and noncommunicating hydrocephalus in the dog other alternative mechanisms would play a significant role in CSF drainage.[3,9,22–24,34,41,43]

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